LAPINONE, A NEW ANTIMALARIAL*

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Lapinone is an antimalarial of novel chemical type and unsual physiological properties. It is a hydroxyalkylnaphthoquinone composed of only carbon, hydogen, and oxygen, and hence it is of simpler composition than all other known antimalarials and, indeed, than any other chemotherapeutic agent.

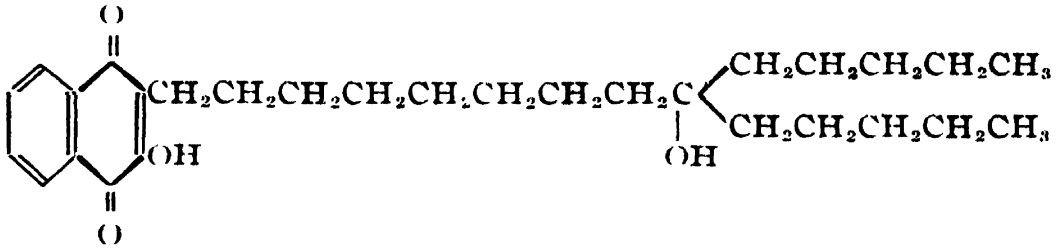

FIG. 1

Quinine is a 6-methoxyquinoline derivative with a nitrogen-containing side chain at position 4. Atabrine, the synthetic substitute suppressive introduced by the I. G. Farbenindustrie (1930), is an acridine derivative, but it is related to quinine in that the first two rings constitute a 6-methoxyquinoline unit with a nitrogen side chain at the 4-position. The other I. G. Farbenindustrie drug plasmochin (1926), now often called pamaquine in the United States, has the atabrine side chain moved to the 8-position of 6-methoxyquinoline ; it is an 8-aminoquinoline derivative. The new American drugs pentaquine (Drake and co-workers, 1946) and primaquine (Elderfield and co-workers, 1946) are related 8-aminoquinolines with small variations from plasmochin in the structure of the side chain ; thus primaquine is simply plasmochin minus the two N-ethyl groups. These 8-aminoquinolines have little suppressive action, but when administered in conjunction with quinine they effect a high percentage of cures of relapsing vivax malaria. Plasmochin is too highly toxic for safe use ; the status of the newer substances in this respect is still uncertain. Among a large number of other compounds synthesized, the Germans prepared the 4-aminoquinolines santochin and the substance now known as chloroquine, but their pharmacologists did not rate the compounds very highly. American research showed that chloroquine is a very effective suppressive and led to

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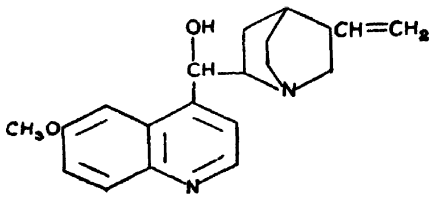
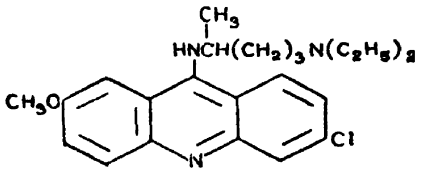
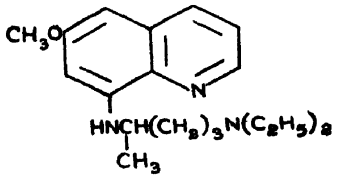
the introdution of the related camoquine (Burckhalter, *et al.*, 1948). The 4-aminoquinolines are suppressive only and lack curative properties. The same is true of the biguanide derivative paludrine (Curd, Davey and Rose, 1945), developed in England by I. C. I. workers.



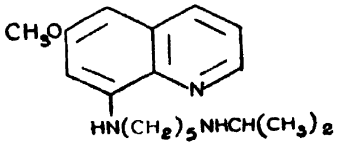
Quinine



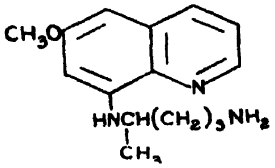
Atabrine



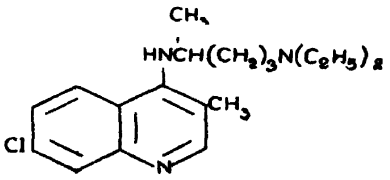
Plasmochin (Pamaquine)



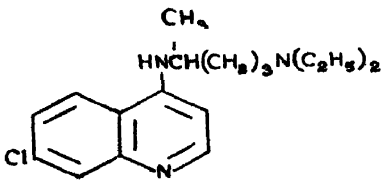
Pentaquine



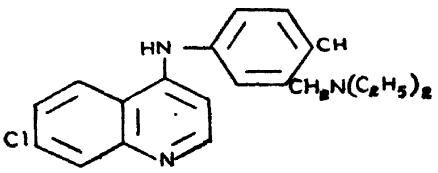
Primaquine



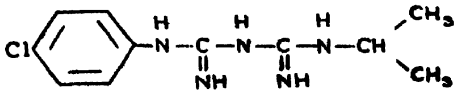
Santochin



Chloroquine



Camoquine



Paludrine

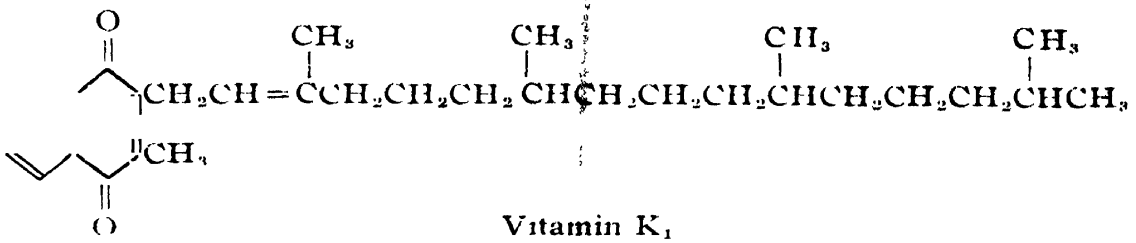
FIG. 2

All of these antimalarials, natural and synthetic, contain nitrogen, and some contain chlorine as well; lapinone contains neither nitrogen nor

chlorine. The sulfa drugs and penicillin are also of elaborate composition, since they contain carbon, hydrogen, oxygen, nitrogen, and sulfur.

Lapinone functions by a mechanism evidently different from that of any other antimalarial. It destroys malarial parasites by inhibiting a respiratory enzyme of parasitized cells. The naphthoquinone seems of particular interest because of indications that it has high potency as both a suppressive and curative drug.

Lapinone resembles vitamin K_1 in elementary composition, structure, and physical properties. Both substances are 1,4-naphthoquinones, and both



Vitamin K_1

FIG. 3

are lipid-soluble, water-insoluble, viscous yellow oils. The antimalarial has at the 2-position a hydroxyl group and at the 3-position a 19-carbon side chain containing a hydroxyl group ; the vitamin has a 2-methyl group and the substituent at position 3 is a 20-carbon side chain containing a double bond.

The development of the new antimalarial resulted from systematic exploitation of a fortuitous clue uncovered in the extensive program of antimalarial research sponsored by the United States government during World War II and organized by the Committee on Medical Research of the Office of Scientific Research and Development. In this program of the C. M. R., some thirty-nine chemical research groups operated under government contract in coordination with pharmacological and clinical research groups. One line of investigation was the synthesis and biological documentation of all conceivable isomers, derivatives, and analogs of the known antimalarials of the quinoline and acridine series ; such work led to the development of camoquine, pentaquine, and primaquine and to recognition of the value of chloroquine. The second line of attack consisted in the biological screening of organic chemicals of a wide variety of types in the search for antimalarials of a new kind. In one such screening program, chemists of the Abbott Laboratories in North Chicago visited some of the larger universities to collect and code miscellaneous research samples and send them to the pharmacological group of Dr. Arthur P. Richardson at the University of Tennessee Medical School at Memphis for screening assays against *Plasmodium lophurae* in ducks. Of several hundred compounds assayed, all but three proved to be completely inactive. The three active compounds, which possessed definite if weak antimalarial activity, were related naphthoquinones that had come from my laboratory at Harvard and that I had taken from

a collection af lapachol samples bequeathed to me by Samuel C. Hooker on his death in 1935. The most interesting of the three compounds was hydrolapachol, the dihydro derivative of lapachol.

Samuel C. Hooker (1889-1896; 1915-1935)

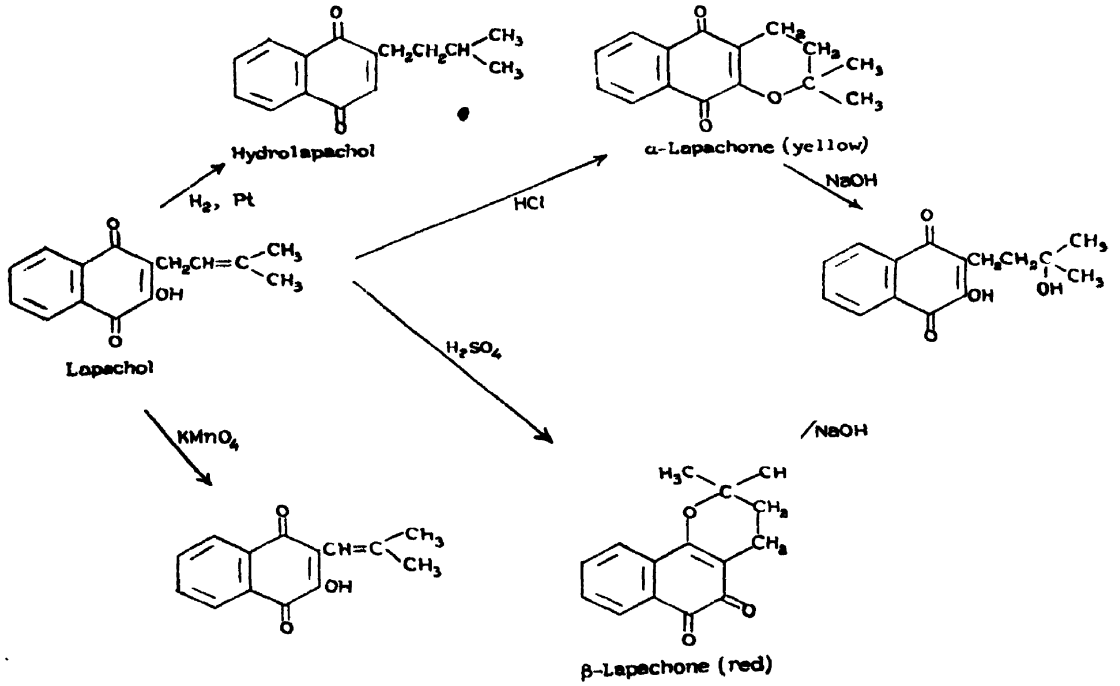


FIG. 4

Lapachol, a yellow pigment that occurs in the grain of certain tropical woods, had been the subject of a remarkable series of researches by the English-born chemist Hooker, a masterful experimentalist whose lot was cast in the field of American sugar technology, in which he achieved brilliant success. A romance with an American girl student at Munich had led Hooker to come to the United States, where, finding no suitable academic posts open, he had accepted employment in a sugar refinery in Philadelphia. Hearing of the presence in the city of a young German-trained chemist, a Philadelphian manufacturer of fine fishing rods and bows and arrows from the rare Bethabarra wood, imported at considerable expense from South America, consulted Hooker about the possible utilization of the yellow pigment of the wood, and provided the young chemist with quantities of Bethabarra sawdust and waste cuttings. Hooker found the chemistry of lapachol a fascinating subject for spare-time research, and in the period 1889-1896 he published a series of brilliant papers reporting the complete elucidation of the structure of lapachol and a description of an amazing array of novel reactions of the interesting substance [Greene and Hooker (1889); Hooker and Greene (1889); Hooker (1892, 1893, 1894, 1896); Hooker and Gray (1893); Hooker and Carnall (1894); Hooker and Walsh (1894); Hooker and Wilson (1894)]. In 1896, although he had not completed the research and had stated in his last paper

that "I shall hope to return to the consideration of this problem in the future" he felt obliged to abandon this side-activlty and devote his whole energies to the professional field of sugar technology. His efforts in this direction were eminently successful ; he introduced the beet sugar industry into American practice and achieved so many other successes that he eventually rose to the post of executive vice-president of his company. But he had not forgotten lapachol, and he was ever conscious of the promise of further work stated in his last paper. So, as soon as he had acquired an adequate competency, Hooker retired from the sugar business in 1915, built a private laboratory in the former stable in back of his elegant residence in Brooklyn, and shortly resumed the lapachol research exactly where he had left off in 1896.

It was my good fortune to meet Dr. Hooker in 1926 and to be in close association with his scientific work until the time of his death. I had applied techniques of oxidation-reduction potential measurements learned in my work for the Doctorate under Professor James B. Conant [Conant, Kahn, Fieser and Kurtz (1922,; Conant and Fieser (1922, 1923, 1924)] to studies of heterocyclic quinones [Fieser (1926) ; Fieser and Ames (1927) ; Fieser and Peters (1931) ; Fieser and Hartwell (1935); Fieser and Kennelly (1935); Fieser and Martin (1935)] and polynuclear quinones, [Fieser (1929;) Fieser and Dietz (1931)] and to an investigation of the tautomerism of hydroxyquinones [Fieser (1928, 1929); Fieser and Fieser (1934); Fieser and Thompson (1939)]. Through Dr. Hooker's generosity in supplying me with samples, I was able to include 16 quinones of the lapachol series in my potentiometric study of tautomerism.

I was fascinated by my contacts with this elderly gentleman who appeared to me to be an extraordinarily gifted experimentalist and a rare personality. Having lost contact with the academic chemists of his own age, Hooker was glad to have a young friend enthusiastically interested in his beloved lapachol chemistry. On his death in 1935, I edited a series of eleven posthumous papers reporting his researches of 1915 to 1935, [Hooker (1936); Hooker and Steyermark (1936); Hooker and Fieser (1936)] and I was bequeathed his extensive collection of beautiful samples of compounds derived from or related to lapachol.

When Richardson's assays revealed antimalarial activity in the relatively simple naphthoquinone hydrolapachol, I was in a particularly favorable position to investigate this new clue. In conducting the potentiometric investigations cited above I had acquired experience with practically all the known methods for the preparation of naphthoquinones and had introduced a few new procedures. Inspired by my association with Dr. Hooker, I had achieved the first synthesis of lapachol. (Fieser, 1927) My student Jesse T. Dunn had synthesized plumbagin in 1936 (Fieser and Dunn, 1936) and I had synthesized vitamin K_1 in 1939 (Fieser, 1939, 1940). My associates and I had developed other syntheses in the naphthoquinone [Fieser, Campbell,

Fry and Gates (1939); Fieser and Weighard (1940); Tishler, Fieser and Wendler (1940); Fieser, Gates and Kilmer (1940); Fieser and Gates (1941); Fieser and Jones (1942); Fieser, Turner (1942); Fieser and Turner (1947)], phenanthrenequinone (Fieser, 1929), anthraquinone (Fieser and Fieser, 1935) and 1,2-benzanthraquinone (Fieser and Dietz, 1929) series, and we had investigated a number of interesting reactions of quinones. [Fieser and Peters (1931, 1935); Fieser (1931); Fieser and Seligman (1934); Fieser and Hartwell (1935); Fieser and Dunn (1936, 1937); Fieser, Hartwell and Seligman (1936); Fieser and Bradsher (1939); Fieser and Fieser (1939, 1941)]. Of particular importance to the problem at hand was the timely discovery with F. C. Chang and A. E. Oxford of the method of peroxide alkylation (Fieser and Chang, 1942; Fieser and Oxford, 1942), whereby hydroxynaphthoquinone is converted into a 2-hydroxy-3-alkyl-1,4-naphthoquinone by reaction in acetic acid solution with the peroxide of an acid. This convenient one-step process provided a quick method for the synthesis of a large number

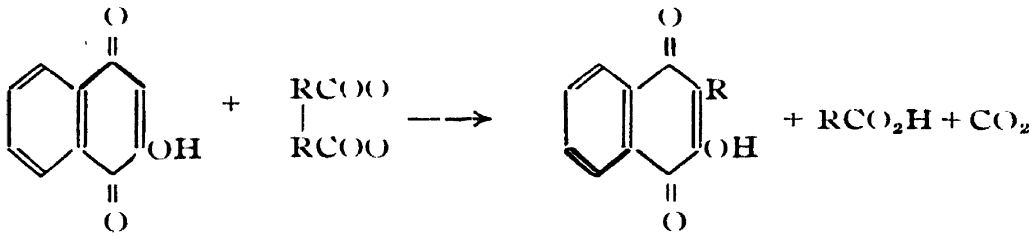


FIG. 5

of substances of the hydrolapachol type having hydrocarbon chains (R) of varying length and including straight chain structures, chains with branches at various points, and chains containing aromatic and alicyclic rings.

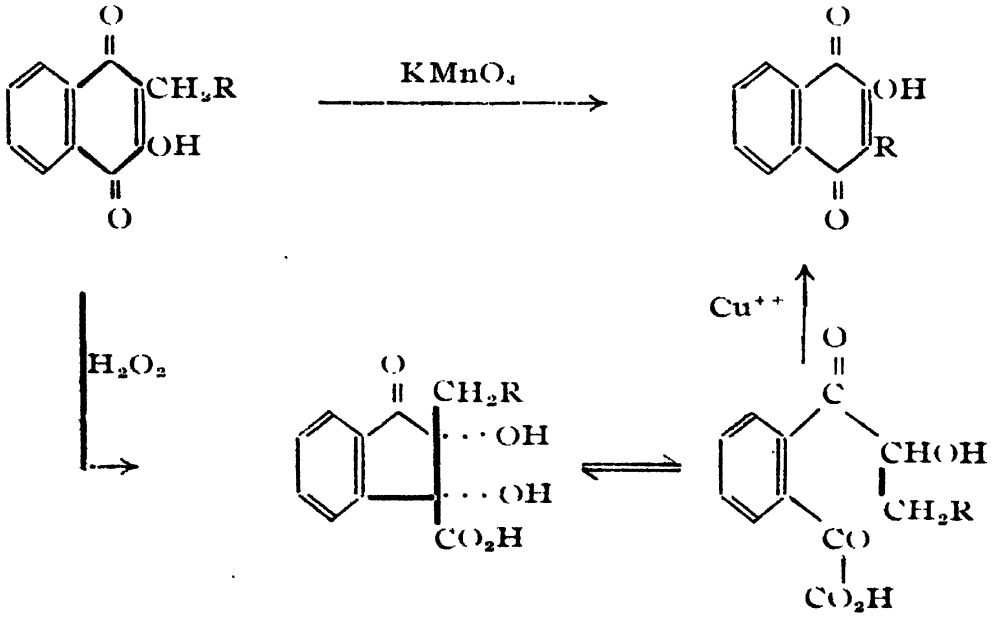


FIG. 6

A second general method that proved of great use to us was a remarkable oxidation discovered by Dr. Hooker, the Hooker oxidation (Hooker, 1936). Hooker had discovered that a hydroxyalkylnaphthoquinone can be converted into the next lower homolog by oxidation with alkaline permanganate in the cold, and he had found that the mysterious disappearance of the methylene group occurs even in the case of lapachol without disturbance of the double bond. He had synthesized the derivative with a seven-carbon side chain and degraded it by seven Hooker oxidations to every one of the lower homologs. The reaction was of great service in our synthetic program; for example, the readily available even-carbon acids afforded, by peroxide alkylation, the series of quinones with C_9, C_{11}, C_{13}, etc. side chains, and Hooker oxidation of the products then yielded the otherwise inaccessible homologs with C_8, C_{10}, C_{12}, etc. side chains. Hartwell, Seligman and I (Fieser, Hartwell and Seligman, 1936) had made a preliminary study of the mechanism of the Hooker oxidation, and in 1940 Mary Fieser and I discovered an intermediate in the reaction and she had subsequently established its structure. When, later, the reaction became of importance to our synthetic program, I worked on the experimental procedure and was able to develop a high-yield two-step process that is applicable to large as well as small quantities and represents a distinct improvement over the original one (Fieser and Fieser, 1948).

My research group, which eventually was expanded to a total of thirteen chemists, plunged into the work of exploring variations in the naphthoquinone structure and synthesized a total of some 250 new quinones. The Abbott Laboratories joined us in the initial synthetic program, and a group of five of their chemists under the direction of Dr. Marlin T. Leffler synthesized an additional 75 quinones for bioassay. The total effort reported in a series of eight joint papers (Fieser *et al*, 1948) included also the preparation of 46 quinone derivatives and of a considerable number of new intermediates. One of my co-workers, Dr. W. G. Dauben, completed some of the synthetic work at the University of California in Berkeley. Another, Dr. Ernst Berliner, spent his summers in Cambridge and continued the synthetic work during term time at Bryn Mawr, with the collaboration of his student Frances Bondhus, now Mrs. Berliner. My Ph. D. students Armin G. Wilson and Evelyn Hodes were married in the course of the work. Finding some periods of respite from my wartime activities in the development of new incendiary munitions, I had the pleasure of personal participation in various phases of the experimental program and worked out procedures for the preparation of several of the key naphthoquinones and intermediates on a practical scale by a diene synthesis (Fieser, 1948).

Interest in the naphthoquinones as antimalarials was greatly enhanced when cooperating workers of the Rockefeller Foundation found that the substances not only suppress malarial infections in chickens but are effective prophylactic agents [Clarke and Theiler, (1948); Whitman, (1948)]. In 1944

these investigations established that the naphthoquinone drugs effectively destroy both the trophozoits of the red blood cells and the exoerythrocytic forms of malaria parasites found in the reticulo-endothelial cells of chickens infected with *Plasmodium gallinaceum*. Quinine and atabrine suppress the trophozoites, but exert no control whatever over the exoerythrocytic forms. These observations strongly suggested that the naphthoquinones would exhibit curative as well as suppressive action, and such an effect was subsequently established in three different avian infections. Walker and Richardson (1948) and Walker, Slanber and Richardson (1948) later observed a very interesting synergistic or potentiating effect of a naphthoquinone and the 8-aminoquinoline derivative plasmochin (pamaquine); a combination of one/tenth the curative dose of each drug resulted in 100% cures.

The program of synthesis by our chemical groups, coupled with prompt and able bioassays by Dr. Richardson's group in Memphis, soon outlined rough relationships between chemical constitution and biological potency (Fieser and Richardson, 1948). We were eventually able to produce compounds possessing no less than one hundred times the potency of hydrolapachol, the compound that had given the principal clue to the new development. The assays for suppressive activity against *Plasmodium lophurae* in ducks were expressed in terms of the effective dose, in mg. of drug per kg. of body weight, required to effect 95% reduction of parasitemia, represented by the symbol ED_{95}. For hydrolapachol, $ED_{95}=68$ mg./kg., and hence the substance is only about one-seventh as active as quinine (base), $ED_{95}=10.25$ mg./kg. The most active naphthoquinone encountered had the high potency $ED_{95}=0.67$ mg./kg., but the compound was available only by a very difficult synthesis.

In 1943 an initial clinical trial was conducted at the Goldwater Memorial Hospital in New York on syphilitic patients undergoing malaria therapy (Wiselogle, 1946). Each cooperating laboratory supplied for the trial the

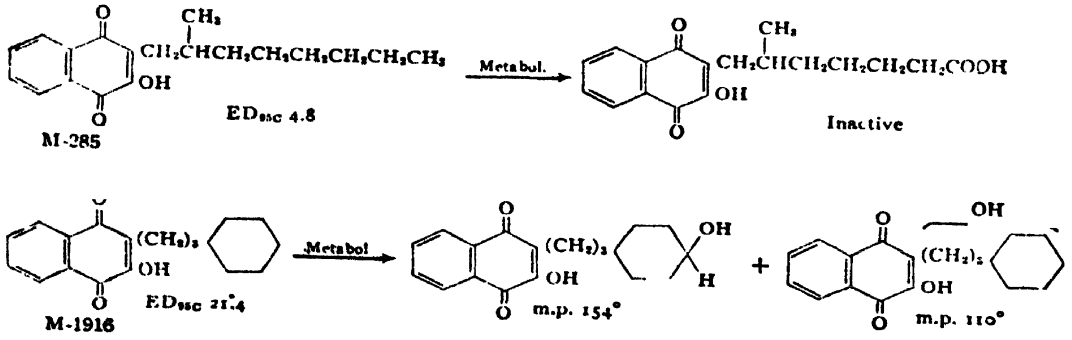


FIG. 7

most promising naphthoquinone that could be produced in a hurry in adequate quantity (250 g.); these were the substances coded as M-285 (Abbott) and M-1916 (Harvard), whose structures and activities are shown in the chart. Although M-285 is four times as potent in the duck assay as M-1916, it proved to be completely inactive in five patients (blood-induced *P. vivax* and

P. falciparum), whereas M-1916 produced a temporary suppression of fever extending for an average of 9.5 days in eight cases. The effect was not satisfactory, but enough to show that M-1916 has definite antimalarial activity in man.

The results were very disappointing. The medical authorities of the CMR felt that the naphthoquinones had been given a fair trial and had failed, and they discouraged us from continuing the study; they pointed to other instances where results in animals fail to carry over to man. The Abbott Laboratories group merely finished off such synthetic work as they had in hand. But my group was not satisfied. We did not like to rest the case without trying to find out why compounds so active in ducks and chickens proved inactive, or only weakly active, in man. We were particularly intrigued with the curious reversal in activity: why is M-285, so potent in ducks, completely inactive in man, whereas M-1916, the weak sister in the duck assays, exerts a mild therapeutic effect in man?

So some of the chemists of my group, particularly Dr. Hans Heymann and Dr. Frederic C. Chang, became biochemists and plunged into the problem of investigating the metabolic fate of naphthoquinones administered to nonmalarial human subjects (Fieser and others, 1948). We soon despaired of the idea of obtaining supplies of urine from the subjects of the clinical trial or of surmounting the governmental red tape involved in obtaining conscientious objectors as subjects, and initiated the study by eating the compounds ourselves. I participated in this phase of the work by consuming a total of 33 g. of various naphthoquinones and working up all the urines and several plasma samples; it was a thrilling experience to have blood drawn from my arm and spun down, and to look at the raspberry red plasma containing metabolites and know that my own liver had accomplished the metabolic oxidations concerned. Later, after returning on VE-day from participation in the Alsos Mission of scientific intelligence that accompanied our armies of conquest in Germany, I enjoyed a period of freedom from both war work and teaching and was able to carry out in the test tube some of the oxidative changes that had occurred in my liver (Fieser, 1948).

After an initial start in which we chemists served as our own guinea pigs, Dr. Arnold M. Seligman came to our aid and provided subjects for study of drug metabolism from the Beth Israel Hospital. He instituted the ingenious practice of administering some of the compounds to polycythemic patients prior to the drawing of the necessarily large volumes of blood in order to provide us with amounts of plasma adequate for isolation of metabolites from this source; in several instances the metabolites extracted from plasma were found to be identical with those excreted in the urine. The required pharmacology was ably done at the Harvard Medical School by Professor Otto Krayer, Dr. E. B. Astwood, and Dr. A. M. Seligman.

In studies that, after expiration of our war-time contract, were continued under a grant from the Rockefeller Foundation, we found that metabolism

of the naphthoquinones in man consists in oxidative attack of the hydrocarbon side chain. Thus the side chain of hydrolapachol, $-CH_2CH_2CH(CH_3)_2$, is metabolized to $-CH_2CH_2C(OH)(CH_3)_2$; the product was identified by comparison with a sample of hydroxyhydrolapachol taken from the Hooker collection. Hooker's samples were of the greatest service in development of colorimetric methods for separating and identifying various alcoholic and unsaturated derivatives of the naphthoquinones (Fieser, 1948).

In all, some eight crystalline products of metabolism were isolated and their structures established. We found that M-285 is metabolized to a carboxylic acid and that M-1916 is metabolized to two hydroxy derivatives, as shown in the formulation above, but the substances were isolable only on a micro scale and not in quantity sufficient for bioassays. We had submitted a few synthetic compounds with oxygenated side chains and they had all been found devoid of activity. "What substance," we kept asking ourselves, "was responsible for the weak therapeutic effect resulting from administration of M-1916?" Dr. W. B. Wendell (1946) at Memphis had discovered that the naphthoquinones are very powerful inhibitors of respiratory enzymes and are effective at dilutions in the order of 1×10^{-6}M. The antirespiratory activity of a given member of the series, relative to that of a reference standard, can be measured on a micro scale in a Warburg apparatus by determining the concentration required to half-inhibit the respiration of a suspension of parasitized red blood cells drawn from a duck infected with *P. lophurae*. By the spring of 1945 Wendell's results so strongly suggested that the convenient *in vitro* determination of antirespiratory activity affords a reliable guide to *in vivo* antimalarial activity that we decided to install the *in vitro* test in our own laboratory for the more precise study of drug metabolism. This meant establishment and maintenance of a colony of malarial infected ducks, housed under some protest in the biological laboratory, and installation and operation of a Warburg apparatus in our own laboratory. The senior chemist of my group, Dr. Hans Heymann, rose to the occasion of mastering the many biological and biochemical techniques involved and ably conducted an extended series of antirespiratory studies, [Heymann and Fieser (1948); Fieser and Heymann (1948)]. Determinations of the relative antirespiratory activities of 76 naphthoquinones in Memphis and of 82 in Cambridge showed conclusively that the *in vitro* test is a safe measure of *in vivo* activity.

The micro test permitted study of the course of metabolic drug deactivation in the following manner. A given compound was administered to a patient by Dr. Seligman, usually intravenously, and small blood samples were drawn from time to time, spun down, and the plasmas sent over to Dr. Heymann in Cambridge. Since oxygenation of the side chain does not alter the chromophoric properties of the naphthoquinone nucleus, colorimetric determination of the pigment extracted from a plasma gave the value of total naphthoquinone, degraded and (or) undegraded. A small sample of pigment

was then put into the Warburg machine and a determination made of the antirespiratory activity per colorimetric equivalent of pigment as compared to that of the drug administered. Naphthoquinones of all of the types initially available proved to be metabolized completely within 3-4 hours. In most cases the initial activity, however high, was completely lost in the process. This was true of M-285, and an *in vitro* test of the carboxylic acid metabolite that we had isolated showed to be completely inactive. The case of M-1916 was different. Here, metabolic change was as rapid as ever but the activity dropped, in four hours, to a level one-tenth that of M-1916 and then persisted at the same level for some twenty hours. The two hydroxylated metabolites that we had isolated were then found, in repeated trials, to possess antirespiratory activity just one-tenth that of M-1916. We thus reached the conclusion that the weak antimalarial action of M-1916 in the clinical trials must have been due to the weakly active but persistent hydroxylated metabolites. A hydroxyl group in the side chain provides protection against metabolic attack but, at least in the case of M-1916 and the few other compounds that we had then investigated, the introduction of a hydroxyl group into the side chain detracts very materially from the potency of the parent compound.

However, our program of systemmatically investigating all types of side chains eventually afforded results suggesting that the deactivating influence of oxygen substitution can be offset by an increase in the carbon content of the side chain. We were later able to rationalize this empirical finding by a physico-chemical study of distribution characteristics. Richardson's assays had shown that in any homologous series, for example hydroxynaphthoquinones with *n*-alkyl-, isoalkyl-, ω-phenylalkyl-, or ω-cyclohexylalkyl-side chains, biological activity increases with increasing molecular weight for a time, passes through a maximum, and then falls off. However, the optimum molecular weight varied considerably from one series to another; in the *n*-alkyl series a C_9-side chain afforded optimum activity, whereas in the ω-cycloalkyl and ω-phenylalkyl series highest potency was reached with C_{10-11} and C_{15}-side chains, respectively. These relationships became clear from a study of the distribution of the naphthoquinones between ether, in which the free acid dissolves with a yellow colour, and aqueous buffers, which extract a certain amount of the red anion (Fieser, Ettlinger and Fawaz, 1943). By colorimetry, it is a simple matter to make an accurate determination of the constant p_R, which characterizes the balance of hydrophilic-lipophilic properties of a given member of the series. This constant is defined (see equation) as the p_H of an aqueous buffer

$$p_R = \log \frac{[\text{Quinone in ether layer}]}{[\text{Quinone in water layer}]} + p_H - 2$$

capable of extracting just 1/101-part of pigment from an equal volume of ether. In each series p_R increases with increasing molecular weight, and the

curves for the different series are parallel. The effect of the introduction of cyclohexyl and phenyl groups into the side chain is to produce progressive shifts in the direction of increased hydrophilic character. These shifts follow closely the displacements in the peaks of antimalarial activity from series to series, and the compounds of highest activity in the different series all have p_H values in the range 10-12. Thus a naphthoquinone, to possess high biological potency, must have a balance between hydrophilic and lipoyhilic properties corresponding to a p_H of this favourable range.

A hydroxyl group in the side chain, desired for protection against metabolic attack, very greatly enhances the hydrophilic character of the molecule; the effect amounts to a displacement in p_H of about 4 units. M-1916 is close to being the peak compound in its series, but the hydroxyl group in the metabolites reduces p_H to an unfaborably low level. However, an appropriate increase in the molecular weight should compensate for the hydrophilic shift caused by the hydroxyl group. We therefore decided to try to synthesize a naphthoquinone with a hydroxyl group substituted in a very large hydrocarbon side chain. At the time this project was taking shape we had the pleasure of the addition to our group of Dr. George Fawaz, on leave of absence in 1945-1946 from the Department of Chemistry of the American University of Beirut, Labanon, and now Professor of Pharmacology at Beirut. Dr. Fawaz, who with the able assistance of his wife Eva, participated in the distribution study cited, undertook the synthesis of a compound of the type specified and, by application of a method developed by Dr. Heymann for the synthesis of a similar model compound, synthesized the compound coded as M-2350, now designated lapinone. He synthesized three related homologs, but they proved less promising. Later, three Ph.D. students synthesized a large number of analogous quinones with hydroxylated side chains, and with side chains containing ketonic, oxide, sulfide, and nitrogen functions [Cram (1949); Paulshock and Moser (1950); Moser and Paulshock (1950)] but most of these compounds proved to be much less potent than lapinone and none seem more promising. The first guess was thus a lucky one.

M-2350, lapinone, was initially assayed by Dr. Richardson by the usual route of oral administration and found to be only moderately active. I thought this might be due to poor absorption from the gut of this compound of high molecular weight and asked for a reassay. Surely enough, intramuscularly administered material proved to possess the high antimalarial potency that we had predicted. We then, hopefully, investigated the resistance of the substance to metabolic deactivation in the human body. Dr. Seligman administered the compound intravenously to patients of the Beth Israel Hospital and Dr. Heymann determined the residual activity of extracted plasma pigments (Fieser, Heymann and Seligman, 1948). The results were very encouraging. Unlike M-1916, M-285, and all the other naphthoquinones with hydrocarbon side chains, lapinone retained a large measure of its original

activity for periods of 20-40 hrs, after administration. The results were substantiated by experiments with animals of seven species. Mice metabolize M-1916 more nearly like humans than do any of the six other test animals studied, and lapinone administered to mice shows good retention of antirespiratory activity.

By the criteria of the laboratory tests, it appeared that the goal of developing a naphthoquinone of high potency and of adequate resistance to metabolic deactivation in man had been achieved. We thus awaited with great interest the outcome of an initial, small scale clinical trial conducted by Dr. Fawaz on his return to the American University at Beirut. Nine patients infected with primary vivax malaria were given 2 g. of lapinone per day for four days by intravenous injection in gelatine solution. All patients were relieved very promptly of fever and the blood was freed of parasites. Thus, unlike M-285 and M-1916, lapinone exerts a fully satisfactory suppressive action in man just as it does in ducks and chickens: The result showed that the defect in the early compounds had been corrected and it justified our sustained effort to solve a problem in chemotherapy through a program of rational research aimed at understanding the fundamental phenomena involved. The Lebanese clinical trial was planned merely as a test for suppressive action in man, and the period of administration was not at all comparable to the 14-day regime employed in the standard test for curative action. Nevertheless, six of the patients treated had gone without relapses when last checked after periods of from thirteen to fifteen months after termination of treatment. The result tentatively suggests a carry-over to man of the curative action of other naphthoquinones observed in birds.

More recently, Dr. Alexander M. Moore of Parke, Davis and Co. has reported results of a reliable new test for supperssive and curative action against *P. lophurae* in white leghorn chicks that he had previously applied to all the standard antimalarials and to several of the newer compounds. In a letter of January 3, 1951, Dr. Moore states that : "As a suppressive, lapinone was about three times as active as quinine, i. e. ED_{95} ca. 5 mg./kg. daily for 4 days, when administered intramuscularly. As a curative agent, lapinone was highly effective when administered intramuscularly at the maximum tolerated dose. Of all the types of antimalarial drugs tested, only the 8-aminoquinolines and the 2-hydroxy-3-alkyl-1, 4-naphthoquinones have cured young chicks infected with *lophurae* malaria. Although the 8-aminoquinolines cured only a fraction of the infected birds, lapinone was much more effective. Indeed, you will observe from the attached reports that lapinone cured every one of the infected birds. No other substance tested in this laboratory to date has proved so effective in the curative test."

Lapinone thus appears promising enough to warrant further pharmacological study and clinical evaluation. A practical synthesis from β-naphthol and sebacic acid was reported in 1950 (Fawaz and Fieser, 1950). Endo Products Inc. of New York has initiated manufacture of lapinone, at least

on a scale adequate for pharmacological and clinical evaluation. Since the subatance is a water-insoluble oil, in our laboratory tests and in the Lebanese trial it was administered by intravenous or intramuscular* injection in order to by-pass the question of efficiency of absorption from the intestines. However, Dr. Nathan Weiner of Endo Products has recently developed a promising formulation for oral administration and is investigating its efficacy.

I hope that some of the many able medical scientists of India will apply their knowledge, experience, and vast test material to the study of this new compound. It would be very gratifying if our own prolonged research, as well as the early fundamental investigations of Dr. Hooker, should turn out to be of some service to the people of this great country, where malaria is still a major problem.

Solution for intravenous injection (developed by Dr. A. M. Seligman): dissolve 2 g. of lapinone in 50 cc. of alcohol and add all at once with agitation a solution prepared from 1500 cc. of physiological saline, 200 cc. of 6% Knox pyrogen-free gelatin solution (*p*H 7.4), and 1.2 g. of sodium carbonate monohydrate; the solution should not be prepared more than a few hours before required for injection.

Solution for intramuscular injection (developed by Dr. George Fawaz): dissolve 5 g. of lapinone in 13 cc. of sterile peanut oil and to the solution add a solution of 0.2 g. of Butescin $H_2NC_6H_4CO_2C_4H_9-p$) in 2 cc. of ether.

REFERENCES

Buckhalter, H. J. and others, 1948, *J Am. Chem. Soc*, **70**, 1363.
Clarke, D. H. and Theiler, M., 1948, *J. Infectious Diseases*, **82**, 138.
Conant, J. B. and Fieser, L. F., 1922, *J. Am. Chem Soc*, **44**, 2480.
,, ,, 1923, *ibid*, **45**, 2194.
,, ,, 1924, *ibid.*, **46**, 1858, 263).
Conant, J. B., Khan, H. M., Fieser, L. F., and Kurtz, S. S. Jr., 1922, *J. Am. Chem. Soc.*, **44**, 1382.
Cram, D. J., 1949, *J. Am. Chem. Soc.*, **71**, 3950, 3953.
Curd, F. H. S., Davey, D C., and Rose, F. L., 1945, *Ann. Trop. Med.*, **39**, 220.
Drake, N. L. *et al*, 1946, *J. Am. Chem. Soc*, **68**, 1529.
Elderfied, R. E. *et al*, 1946, *ibid.*, **68** 1524.
Fawaz, G. and Fieser, L F, 1950, *J. Am. Chem Soc*, **72**, 996.
Fieser, L. F., 1926, *J Am Chem. Soc.*, **48**, 1077
,, ,, 1927, *J. Am. Chem Soc.*, **49**, 857.
,, ,, 1928, *ibid*, **50**, 439
,, ,, 1929, *ibid.*, **51**, 3101.
,, ,, 1929, *ibid*, **51**, 940, 1896, 1935.
,, ,, 1931, *Ber.*, **64**, 701.
,, ,, 1931, *J. Am. Chem. Soc.*, **53**, 2329.
,, ,, 1939, *ibid.*, **61**, 2559, 2562, 3467

Fieser, L. F., 1940, *J. Biol. Chem.*, **133**, 391.
,, ,, 1948, *J. Am. Chem. Soc.*, **70**, 3165.
,, ,, 1948, ,, ,, 3237.
,, ,, 1948, ,, ,, 3234.
,, ,, and Ames, M.A, 1927, *J. Am. Chem. Soc.*, **49**, 2604.
,, ,, and Bradsher, C. K., 1939, *ibid*, **61**, 417.
,, ,, and Campbell, W. P., Fry, E. M., Gates, M. D., Jr., 1939, *J. Am. Chem. Soc.*, **61**, 3216.
,, and Chang, F. C., 1942, *ibid*, **64**, 2045.
,, ,, ,, ,, Dauben, W. G., Heidelberger, C., Heymann, H. and Seligmen, A. M., 1948, *J. Pharm. Exp. Therap.*, **94**, 85.
,, ,, and Deitz, E. M., 1931 *J. Am. Chem. Soc.*, **53**, 1128.
,, ,, ,, ,, ,, 1929, *ibid*, **51**, 3141.
,, ,, and Dunn, J. T., 1936, *ibid.*, **58**, 1054.
,, ,, ,, ,, ,, 1936, *ibid.*, **58**, 572.
,, ,, ,, ,, ,, 1937, *ibid.*, **57**, 1016, 1021, 1024.
,, ,, Ettlinger, M. G., and Fawaz, G., 1948, *ibid.*, **70**, 3228.
,, ,, and Fieser, M., 1934, *ibid.*, **56**, 1565.
,, ,, ,, ,, 1935, *ibid.*, **57**, 1679.
,, ,, ,, ,, 1939, *ibid.*, **61**, 595.
,, ,, ,, ,, 1941, *ibid.*, **63**, 1572.
,, ,, ,, ,, 1948, *ibid.*, **70**, 3215.
,, ,, and Gates, M. D., Jr., 1941, **63**, 2948.
,, ,, ,, ,, ,, and Kilmer, G. W., 1940. *ibid.*, **62**, 2966.
,, ,, and Hartwell, J. L., 1935, *ibid.*, **57**, 1479
,, ,, ,, ,, ,, 1935, *ibid.*, **57**, 1479, 1482, 1484.
,, ,, Hartwell, J. L., and Seligman, A. M., 1936, *ibid.*, **58**, 1223.
,, ,, and Heymann, H., 1948, *J. Biol. Chem.*, **176**, 1363
,, ,, Heymann, H., and Seligman, A. M., 1948, *J. Pharm. Exp. Therap.*, **94**, 112.
,, ,, and Jones, R. N., 1942, *J. Am. Pharm. Assoc.*, **31**, 315.
,, ,, and Kennelly, R. G., 1935, *J. Am. Chem. Soc.*, **57**, 1611.
,, ,, Leffeler, M. T., and co-workers, 1948, *ibid.*, **70**, 3174, 3181. 3185, 3195. 3197, 3203, 3206, 3212.
,, ,, and Martin, E. L., 1935, *ibid*, **57**, 1835, 1840, 1844.
,, ,, and Oxford, A. E., 1942, *ibid*, **64**, 2060.
,, ,, and Peters, M. A., 1931, *J. Am. Chem. Soc.*, **53**, 4080, 793.
,, ,, 1935, *ibid.*, **57**, 491.
,, ,, and Richardson, A. P., 1948, *ibid.*, **70**, 3156.
,, ,, and Seligman, A. M., 1934, *ibid.*, **56**, 2690.
,, ,, and Thompson, H. T , 1939, *ibid.*, **61**, 376.
,, ,, Tishler, M. and Wendler, N. L., 1940, *ibid*, **62**. 2861.
,, ,, and Turner, R. B , 1947, *ibid.*, **69**, 2335, 2338
,, ,, and Weighard, C. W., 1940, *ibid.*, **62**, 153.
Greene, W. H., and Hooker, S. C., 1889, *Am Chem. J.*, **11**, 267.
Heymann, H and Fieser, L. F., 1948, *J. Pharm. Exp. Therap.*, **94**, 97.
,, ,, ,, ,, ,, 1948, *J. Biol. Chem.*, **176**, 1359.
,, ,, and Seligman, A. M., 1948, *J. Pharm. Exp. Therap.*, **94**, 85.
Hooker, S. C., 1892, *J. Chem Soc.*, **61**, 611.
,, ,, 1893, ,, ,, **63**, 1376.
,, ,, 1894, ,, ,, **65**, 15.

Hooker, S. C., 1896, *J. Chem. Soc.*, **69**, 1355, 1381.
,, ,, 1936, *J. Am. Chem. Soc.*, **58**, 1163, 1168, 1174, 1181, 1190, 1212, 1179.
,, ,, and Carnell, W. C., 1894, *J. Chem. Soc.*, **65**, 76.
,, ,, and Fieser, L. F. 1936, *J. Am Chem. Soc.*, **58**, 1216.
,, ,, and Grey, A. D., 1893, *J. Chem. Soc.*, **63**, 424.
,, ,, and Greene, W. H., 1889, *Am. Chem. J.*, **11**, 393.
,, ,, ,, ,, 1889, *idem., Ber.*, **22**, 1723.
,, ,, and Steyermark, A B., 1936, *J. Am. Chem Soc.*, **58**, 1179, 1198, 1202, 1207.
,, ,, and Walsh, J. G., Jr., 1894, *J. Chem. Soc.*, **65**, 321.
,, ,, and Wilson, E., 1894, *ibid.*, **65**, 717.
Moser, C. M., and Paulshock. M , 1950, *J. Am. Chem. Soc.*, **72**, 5419.
Paulshock, M., and Moser, C. M. 1950, *J. Am. Chem. Soc.*, **72**, 5073.
Tishler, M., Fieser, L. F. and Wendler, N. L., 1940, *ibid* , **62**, 1982, 2856.
Walker, H. A., and Richardson, A. P., 1948, *J. Nat. Mal. Soc.*, **7**, 4,
Walker, H. A., Stauber, L. A. and Richardson, A. P., 1948, *J. Infectious diseases.*, **82**, 187.
Wendell, W. B , 1946, *Federation Proc* , **5**, 406.
Whitman, L , 1948, *J. Infectious Diseases*, **82**, 251.
Wiselogle, F. Y., 1946, *Survey of Antimalarial Drugs.*